

## F STABILITY STUDY AT $-80^{\circ}\text{C}$ AND $-20^{\circ}\text{C}$

o observe any change in LIF over 5 months stored at  $-80^{\circ}\text{C}$  and  $-20^{\circ}\text{C}$

### Solutions

#### HPLC Buffers

RP-HPLC (A) 0.1% trifluoroacetic acid  
(B) 70% acetonitrile / 0.1% trifluoroacetic acid  
(see 23/1/97)

IEC (A) 50mm phosphate buffer pH 7.0  
(B) 50mm phosphate buffer pH 7.0 / 1M NaCl  
(see 2/1/97)

SEC 300mm phosphate buffer pH 7.2  
(see 31/1/97)

#### Sample Buffers

Acetate buffer (11.22mm)

11.22mm NaAcetate 5.61% sorbitol 0.011% tween 80

11.22mm Acetic acid 5.61% sorbitol 0.011% tween 80

→ mix to pH 3.96 pH 4.45

NaAcetate 0.382 g / 250ml

sorbitol 14.025g / 250ml

tween 137.5  $\mu\text{l}$  / 250ml of 20% tween 80

Acetic acid 325  $\mu\text{l}$  / 500ml

sorbitol 28.05g / 500ml

tween 275  $\mu\text{l}$  / 500ml of tween 80 20%

Citrate Buffer (11.22mm)

11.22mm Na Citrate 5.61% sorbitol 0.011% tween 80

11.22mm Citric Acid 5.61% sorbitol 0.011% tween 80

→ mix to pH 4.97

Na Citrate 1.65g / 500ml

sorbitol 28.05g / 500ml

tween 275  $\mu\text{l}$  / 500ml of 20% tween 80

275

Citric Acid 1.18g / 500ml  
sorbitol 28.05g / 500ml  
tween 350 $\mu$ l / 500ml of 20% tween 80

### Acetate Buffer (13.75mm)

13.75mm Na Acetate 6.87% sorbitol, 0.014% twe

13.75mm Acetic acid 6.87% sorbitol, 0.014% twe

→ mix to pH 4.46

Na Acetate 0.936g / 500ml  
sorbitol 34.35g / 500ml  
tween 350 $\mu$ l / 500ml of 20% tween 80

Acetic Acid (glacial) 400 $\mu$ l / 500ml

sorbitol 34.35g / 500ml

tween 350 $\mu$ l / 500ml of 20% tween 80

### Citrate Buffer (13.75mm)

13.75mm Na Citrate 6.87% sorbitol, 0.014% twe

13.75mm Citric Acid 6.87% sorbitol, 0.014% twe

→ mix to pH 4.91

Na Citrate 2.02g / 500ml  
sorbitol 34.35g / 500ml  
tween 350 $\mu$ l / 500ml of 20% tween 80

Citric Acid 1.44g / 500ml

sorbitol 34.35g / 500ml

tween 350 $\mu$ l / 500ml of 20% tween 80

### HPLC

#### RP-HPLC

flow 0.3ml/min

wavelength 210nm

injection vol 10 $\mu$ l

run time 55 min

gradient 50% method

IEC flow 1ml/min  
 wavelength 280nm  
 injection vol. 100ul  
 run time 40min  
 gradient IEC method

SEC flow 0.5ml/min  
 wavelength 210nm  
 injection vol 10ul  
 run time 40min  
 gradient SEC method

### FLUSH METHODS

### END METHODS

RPFLS - 100% A for 20min  
 1min to 100% B  
 100% B for 10min  
 1min to 100% A  
 100% A for 28min

RPEND - 1min to 100% A  
 100% A for 14min  
 20min to 100% C  
 flow to 0.1 in 5s

SECFLS - 100% A for 120min

SECEND - 20min to 100% C  
 115min at 0.5ml  
 2min to 0.1ml

IECFLS - 100% A for 10min  
 1min to 100% B  
 10min 100% B  
 1min to 100% A  
 100% A for 23min

IECEND - 2min to 0.3ml/min  
 20min to 100% C  
 1min to 0.2ml

### Sample Table Setup:

flush method (rpfls, iecfls, secfls)  
 sample set methods (sdz, iec, sec)  
 end method (rpend, secend, sece)

Method:

(Non-sterile)

## LIF STABILITY STUDY AT -80°C AND -20°C

- Add 28.51 ml of buffer - 11.22 mM Acetate pH4  
11.22 mM Acetate pH 4.5  
11.22 mM Citrate pH 5

Add 23.28 ml of buffer - 13.75 mM Acetate pH 4.5  
13.75 mM Citrate pH 5

to 5 sterile 50 ml tubes
- Thaw 1 x 60 mg and 9 x 5 mg of stock LIF = 105 mg = 28.61 ml (32ml)

Add the LIF from the 5mg vials to the 60mg LIF in the 50 ml tube using a 20 ml syringe with an 18g needle.
- Add LIF to each tube - 3 x 3ml with 10ml graduated pipette then 3 x 490µl with pipetman for 0.4 mg/ml  
- 8ml 2x with 10ml graduated pipette then 3 x 720µl with pipetman for 1.0 mg/ml

	LIF	Buffer	Total
0.4 mg/ml	3.49 ml	28.51 ml	32 ml
1.0 mg/ml	8.72 ml	23.28 ml	32 ml
total	27.91 ml		

The remaining 0.7 ml of LIF will be used in the preparation of standards.

- Measure and record the pH of each formulation.

Formulation	Theoretical pH	Measured pH
0.4mg/ml LIF/Acetate	4.00	3.95
0.4mg/ml LIF/Acetate	4.50	4.48
0.4mg/ml LIF/Citrate	5.00	4.94
1.0mg/ml LIF/Acetate	4.50	4.47
1.0mg/ml LIF/Citrate	5.00	4.96

(Sterile)

Procedure for sterile work:

1. Swab laminar flow hood with ethanol
2. Turn U.V. on for 30 min before use
3. Turn U.V. off and the fan on for use
4. Swab everything that is used in the hood with ethanol before placing it inside
5. Wear gowns, booties, hair covers and glasses
6. Wash gloved hands with antiseptic
7. Cover hands and sleeves with ethanol everytime you take them out of the hood
8. Don't pass anything over the top of sterile vials, beakers or solutions.
9. when finished, swab hood with alcohol and put U.V. on for 10 min.

5. Sterile filter each formulation with a 20ml syringe into another sterile 50ml tube, discard the first 1.15 ml through the filter into a vial and cap and label it.
6. For each formulation draw up 12.5 ml in the multi-dispensing pipette and deliver 1.0 ml to 25 vials. Remove the 12.5 ml tip and put the 2.5 ml tip on and draw up 2.5 ml. Add 150 $\mu$ l to each of the 25 vials.
7. Cap, crimp and label all the vials. Put aside 2 vials from each formulation for Time 0 analysis.
8. Place racks containing remaining vials in plastic bags and put half the vials at -20°C and half at -80°C.
9. Analysis:

	Blank
	Standards 0.2, 0.4, 0.7, 1.0 mg/ml
0.4 mg/ml	Acetate pH 4 (2vials) -80°C
	Acetate pH 4 (2vials) -20°C
	Acetate pH 4.5 (2vials) -80°C
	Acetate pH 4.5 (2vials) -20°C
	Citrate pH 5 (2vials) -80°C
	Citrate pH 5 (2vials) -20°C
1.0 mg/ml	Acetate pH 4.5 (2vials) -80°C
	Acetate pH 4.5 (2vials) -20°C
	Citrate pH 5 (2vials) -80°C
	Citrate pH 5 (2vials) -20°C
	Blank
	Standards 0.2, 0.4, 0.7, 1.0 mg/ml

# Results:

## Osmolarity (Freezing Point Depression)

- measured on

SAMPLE	Nº	Osm 1	Osm 2
LIF 0.4mg/ml Acetate pH4	1	291	294
	2	294	300
LIF 0.4mg/ml Acetate pH4.5	1	296	299
	2	294	294
LIF 0.4mg/ml Citrate pH5	1	303	304
	2	304	303
LIF 1.0mg/ml Acetate pH4.5	1	299	293
	2	290	294
LIF 1.0mg/ml Citrate pH5	1	314	308
	2	306	301
Reference : 301mOsm			

## Buffer osmolality

11.22mm Acetic acid	324	13.75mm Acetic acid	399
11.22mm Na Acetate	332	13.75mm Na Acetate	408
11.22mm Citric acid	323	13.75mm Citric acid	403
11.22mm Na Citrate	346	13.75mm Na Citrate	433

## pH

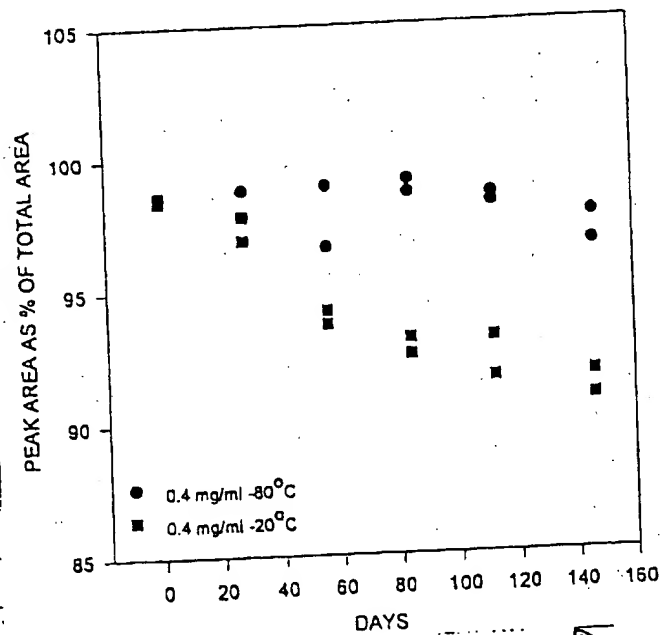
SAMPLE	Nº	INITIAL	Day 28	Day 56	Day 84	DAY 112	DAY 14
AC4 0.4 -20°	1	3.95	3.94	4.03	4.03	4.05	3.91
	2		3.96	4.04	4.02	4.06	3.92
AC4.5 0.4 -20°	1	4.48	4.47	4.53	4.53	4.57	4.43
	2		4.47	4.52	4.53	4.56	4.43
CIT 5 0.4 -20°	1	4.94	4.95	4.96	4.97	5.00	4.95
	2		4.95	4.96	4.97	4.99	4.96
AC4.5 1.0 -20°	1	4.47	4.49	4.50	4.50	4.55	4.43
	2		4.47	4.50	4.51	4.55	4.43
CIT 5 1.0 -20°	1	4.96	4.96	4.94	4.96	4.99	4.95
	2		4.96	4.94	4.96	4.98	4.94
AC4 0.4 -80°C	1	3.95	3.98	3.98	4.05	4.06	3.83
	2		4.00	3.99	4.06	4.07	3.82
AC4.5 0.4 -80°C	1	4.48	4.50	4.49	4.56	4.58	4.38
	2		4.49	4.58	4.54	4.57	4.39

SAMPLE	Nº	INITIAL	DAY 28	DAY 56	DAY 84	DAY 112	DAY 146
CITS 0.4 -80°C	1	4.94	4.98	4.99	5.00	5.01	4.92
	2		4.98	4.98	5.00	5.01	4.92
CITS 1.0 -80°C	1	4.47	4.47	4.51	4.53	4.56	4.40
	2		4.46	4.50	4.54	4.55	4.39
CITS 1.0 -80°C	1	4.96	4.97	4.95	4.98	4.99	4.93
	2		4.97	4.95	4.97	4.99	4.93

### Particle Size

Particle size was measured for each sample at -20° & -80° on Day 56 after thawing on a malvern Instruments Zetasizer 3000 at 25°C. All samples had 0 particles present.

### LIF IN ACETATE BUFFER pH 4.0

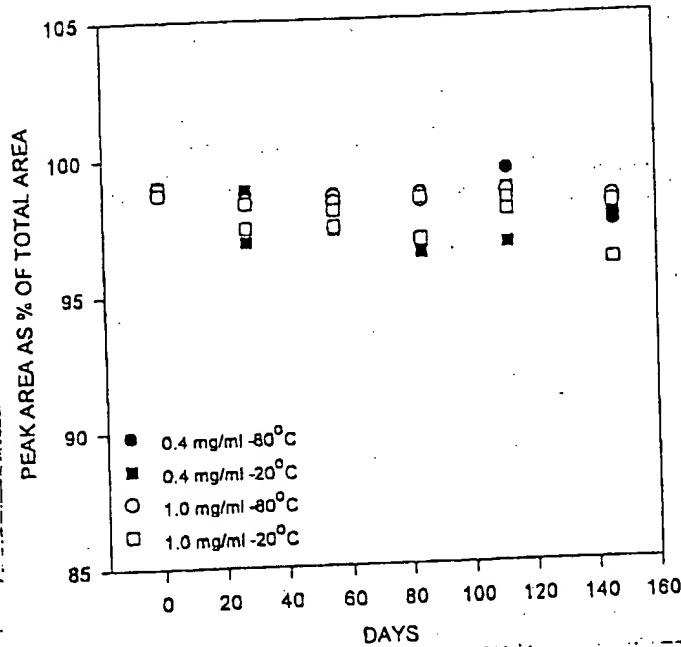


IEC

Table 2. Summary of 0.4 mg/ml, pH 4.0 LIF Stability Following Storage at -80°C and -20°C

measured pH	buffer	Nominal LIF Conc. (mg/ml)	Storage Temp. (C)	Storage Time (days)	RP - Measured Conc. (mg/ml)	RP - Main Peak (area %)	IEC - Measured Conc. (mg/ml)	IEC - Main Peak (area %)	SEC - Measured Conc. (mg/ml)	SEC - Main Peak (area %)
3.95	acetate	0.4	-80	0	0.41, 0.40	100, 100	0.38, 0.38	98.4, 98.6	0.40, 0.40	98.9, 98.5
3.98				28	0.41, 0.41	100, 100	0.38, 0.39	97.8, 98.8	0.39, 0.40	98.2, 98.0
3.99				56	0.41, 0.41	100, 100	0.37, 0.38	98.6, 98.9	0.39, 0.39	98.3, 98.3
4.05				84	0.43, 0.42	100, 100	0.40, 0.39	98.6, 99.1	0.41, 0.41	98.3, 98.8
4.06				112	0.40, 0.40	100, 100	0.38, 0.38	98.2, 98.5	0.39, 0.39	97.9, 97.9
3.88				146	0.42, 0.42	100, 100	0.38, 0.39	98.6, 97.7	0.40, 0.40	98.0, 98.0
3.95	acetate	0.4	-20	0	0.41, 0.40	100, 100	0.38, 0.38	98.4, 98.6	0.40, 0.40	98.9, 98.5
3.95				28	0.41, 0.42	100, 100	0.38, 0.39	96.9, 97.8	0.40, 0.40	98.7, 98.5
4.04				56	0.40, 0.41	100, 100	0.38, 0.38	94.2, 93.7	0.40, 0.40	99.0, 98.9
4.02				84	0.42, 0.43	100, 100	0.38, 0.38	92.5, 93.1	0.42, 0.41	99.2, 98.9
4.05				112	0.40, 0.40	100, 100	0.37, 0.34	93.1, 91.6	0.39, 0.39	98.8, 98.5
					0.41, 0.42	100, 100	0.38, 0.36	90.8, 91.7	0.40, 0.40	98.7, 98.7

LIF IN ACETATE BUFFER pH 4.5



IEC

Table 4. Summary of 0.4 mg/ml, pH 4.5 LIF Stability Following Storage at -80°C and -20°C

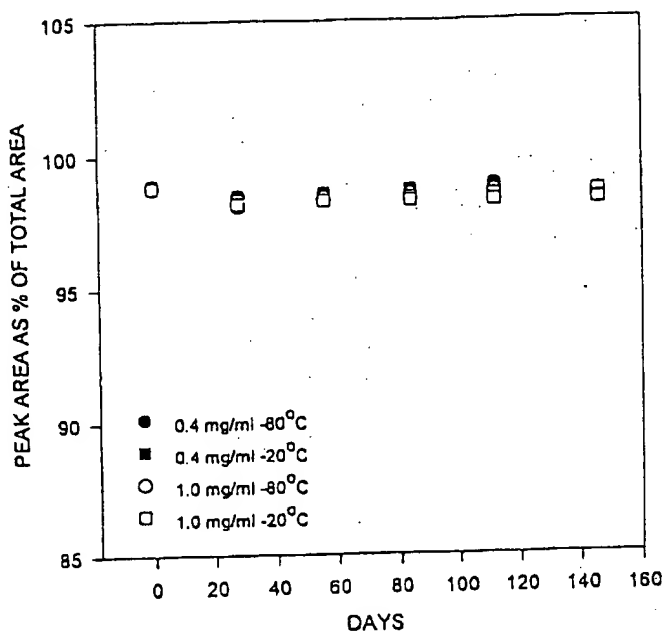
measured pH	buffer	Nominal LIF Conc. (mg/ml)	Storage Temp. (C)	Storage Time (days)	RP - Measured Conc. (mg/ml)	RP - Main Peak (area %)	IEC - Measured Conc. (mg/ml)	IEC - Main Peak (area %)	SEC - Measured Conc. (mg/ml)	SEC - Main Peak (area %)
4.48	acetate	0.4	-80	0	0.40, 0.40	100, 100	0.39, 0.40	99.0, 98.9	0.40, 0.40	98.9, 98.8
4.49				28	0.41, 0.40	100, 100	0.39, 0.38	98.7, 98.7	0.40, 0.39	98.1, 98.0
4.49				56	0.40, 0.40	100, 100	0.38, 0.38	98.5, 98.6	0.39, 0.39	98.4, 98.2
4.54				84	0.42, 0.42	100, 100	0.40, 0.40	98.8, 98.4	0.42, 0.42	98.5, 98.5
4.57				112	0.40, 0.41	100, 100	0.38, 0.39	99.4, 98.6	0.39, 0.39	98.1, 98.0
4.39				148	0.42, 0.42	100, 100	0.39, 0.38	97.4, 97.6	0.40, 0.40	98.1, 98.2
4.48	acetate	0.4	-20	0	0.40, 0.40	100, 100	0.39, 0.40	99.0, 98.9	0.40, 0.40	98.9, 98.8
4.47				28	0.41, 0.41	100, 100	0.39, 0.38	98.8, 96.9	0.40, 0.40	98.4, 98.6
4.52				56	0.40, 0.40	100, 100	0.39, 0.38	98.5, 97.3	0.40, 0.39	98.6, 98.7
4.53				84	0.42, 0.42	100, 100	0.40, 0.40	96.4, 96.5	0.42, 0.42	99.0, 99.0
4.58				112	0.40, 0.40	100, 100	0.39, 0.38	98.7, 98.7	0.39, 0.39	98.2, 98.4
4.43				148	0.42, 0.42	100, 100	0.39, 0.39	97.8, 97.8	0.40, 0.40	98.4, 98.4

Table 6. Summary of 1.0 mg/ml, pH 4.5 LIF Stability Following Storage at -80°C and -20°C

measured pH	buffer	Nominal LIF Conc. (mg/ml)	Storage Temp. (C)	Storage Time (days)	RP - Measured Conc. (mg/ml)	RP - Main Peak (area %)	IEC - Measured Conc. (mg/ml)	IEC - Main Peak (area %)	SEC - Measured Conc. (mg/ml)	SEC - Main Peak (area %)
4.47	acetate	1.0	-80	0	1.00, 1.00	100, 100	0.97, 0.98	98.9, 98.7	0.99, 0.99	98.7, 98.6
4.47				28	1.00, 1.00	100, 100	0.97, 0.97	98.4, 98.3	0.99, 0.99	98.4, 98.4
4.50				56	0.99, 0.98	100, 100	0.96, 0.95	98.5, 98.3	0.97, 0.97	98.5, 98.6
4.53				84	1.00, 1.00	100, 100	0.96, 0.96	98.3, 98.5	0.98, 0.98	98.8, 98.4
4.55				112	1.00, 1.00	100, 100	0.98, 0.96	98.6, 98.6	0.99, 0.99	98.4, 98.5
4.40				146	1.03, 1.02	100, 100	0.95, 0.95	98.3, 98.1	0.99, 0.99	98.4, 98.5
4.47	acetate	1.0	-20	0	1.00, 1.00	100, 100	0.97, 0.98	98.9, 98.7	0.99, 0.99	98.7, 98.6
4.48				28	1.00, 0.99	100, 100	0.98, 0.97	98.3, 97.4	1.00, 0.99	98.5, 98.6
4.50				56	0.98, 0.99	100, 100	0.94, 0.96	97.4, 98.0	0.98, 0.98	98.8, 98.5
4.50				84	0.99, 0.99	100, 100	0.95, 0.97	96.9, 98.4	0.99, 0.99	98.7, 98.5
4.55				112	1.00, 1.00	100, 100	0.97, 0.96	98.3, 97.9	0.97, 0.97	98.4, 98.5
4.43				146	1.02, 1.03	100, 100	0.96, 0.94	98.1, 98.0	0.99, 0.99	98.4, 98.6



## LIF IN CITRATE BUFFER pH 5.0



IEC

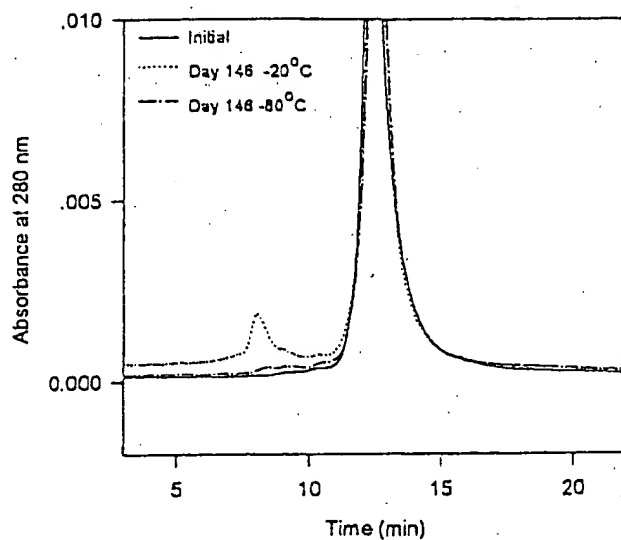
Table 8. Summary of 0.4 mg/ml, pH 5.0 LIF Stability Following Storage at -80°C and -20°C

measured pH	buffer	Nominal LIF Conc. (mg/ml)	Storage Temp. (°C)	Storage Time (days)	RP - Measured Conc. (mg/ml)	RP - Main Peak (area %)	IEC - Measured Conc. (mg/ml)	IEC - Main Peak (area %)	SEC - Measured Conc. (mg/ml)	SEC - Main Peak (area %)
4.94	citrate	0.4	-80	0	0.41, 0.40	100, 100	0.40, 0.40	98.9, 98.8	0.39, 0.39	98.8, 98.7
4.98				28	0.41, 0.41	100, 100	0.39, 0.39	98.5, 98.5	0.39, 0.39	98.3, 98.3
4.98				58	0.40, 0.40	100, 100	0.38, 0.38	98.6, 98.6	0.38, 0.38	98.4, 98.3
5.00				84	0.42, 0.42	100, 100	0.40, 0.40	98.7, 98.4	0.41, 0.41	98.5, 98.5
5.01				112	0.40, 0.40	100, 100	0.38, 0.38	98.9, 98.8	0.39, 0.38	98.3, 98.3
4.92				146	0.41, 0.41	100, 100	0.39, 0.39	98.5, 98.6	0.39, 0.39	98.3, 98.2
4.94	citrate	0.4	-20	0	0.41, 0.41	100, 100	0.40, 0.40	98.9, 98.8	0.39, 0.39	98.8, 98.7
4.95				28	0.41, 0.41	100, 100	0.39, 0.39	98.5, 98.5	0.40, 0.40	98.5, 98.6
4.96				58	0.40, 0.40	100, 100	0.38, 0.39	98.4, 98.6	0.39, 0.39	98.6, 98.6
4.97				84	0.42, 0.42	100, 100	0.41, 0.41	98.6, 98.7	0.41, 0.41	99, 98.8
5.00				112	0.40, 0.39	100, 100	0.39, 0.39	98.7, 98.6	0.39, 0.39	98.5, 98.5
4.95				146	0.42, 0.42	100, 100	0.39, 0.39	98.5, 98.4	0.40, 0.40	98.5, 98.4

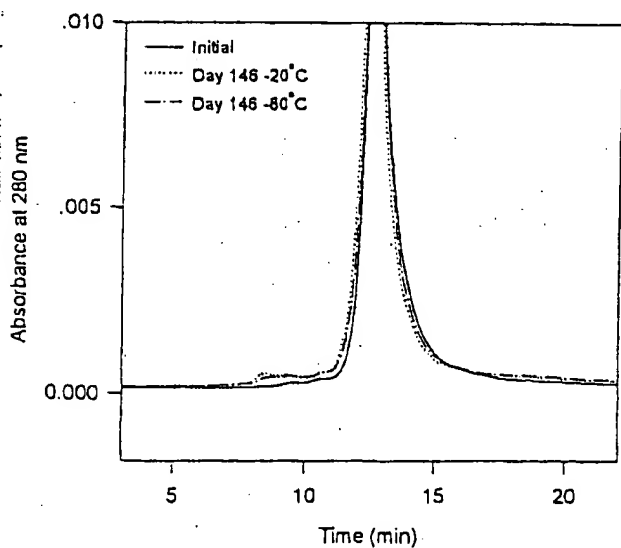
Table 10. Summary of 1.0 mg/ml, pH 5.0 LIF Stability Following Storage at -80°C and -20°C

measured pH	buffer	Nominal LIF Conc. (mg/ml)	Storage Temp. (°C)	Storage Time (days)	RP - Measured Conc. (mg/ml)	RP - Main Peak (area %)	IEC - Measured Conc. (mg/ml)	IEC - Main Peak (area %)	SEC - Measured Conc. (mg/ml)	SEC - Main Peak (area %)
4.98	citrate	1.0	-80	0	1.00, 1.00	100, 100	0.98, 0.99	98.8, 98.8	0.98, 0.98	98.1, 98.1
4.97				28	1.00, 0.99	100, 100	0.96, 0.96	98.2, 98.1	0.98, 0.98	98.4, 98.4
4.95				58	0.97, 0.97	100, 100	0.95, 0.95	98.4, 98.4	0.96, 0.96	98.5, 98.4
4.98				84	0.99, 0.99	100, 100	0.96, 0.96	98.4, 98.5	0.98, 0.97	98.6, 98.5
4.99				112	1.00, 1.00	100, 100	0.96, 0.96	98.6, 98.3	0.98, 0.98	98.5, 98.5
4.93				146	1.03, 1.02	100, 100	0.95, 0.95	98.4, 98.4	0.99, 0.99	98.5, 98.5
4.96	citrate	1.0	-20	0	1.00, 1.00	100, 100	0.98, 0.98	98.8, 98.8	0.98, 0.98	98.1, 98.1
4.98				28	0.99, 1.00	100, 100	0.97, 0.97	98.3, 98.2	0.98, 0.98	98.5, 98.4
4.94				56	0.98, 0.97	100, 100	0.95, 0.95	98.3, 98.3	0.97, 0.96	98.6, 98.5
4.96				84	0.99, 0.99	100, 100	0.96, 0.96	98.4, 98.3	0.98, 0.97	98.4, 98.5
4.99				112	0.99, 1.00	100, 100	0.96, 0.96	98.5, 98.3	0.98, 0.98	98.5, 98.5
4.95				146	1.02, 1.03	100, 100	0.95, 0.95	98.6, 98.3	0.97, 0.98	98.4, 98.4

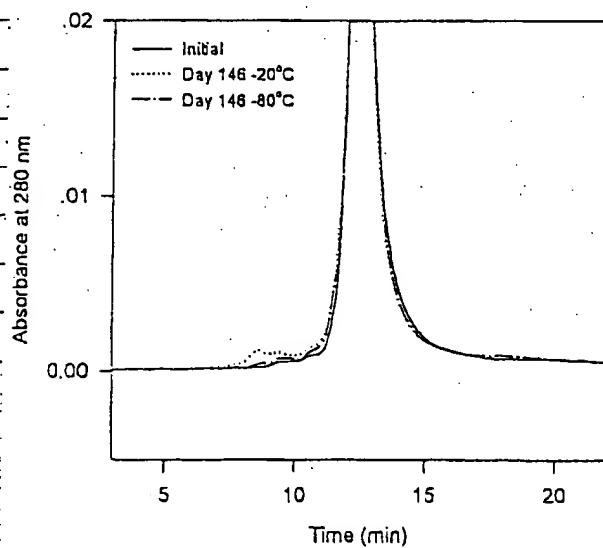
0.4 mg/ml AM424 in pH 4.0 Acetate Buffer



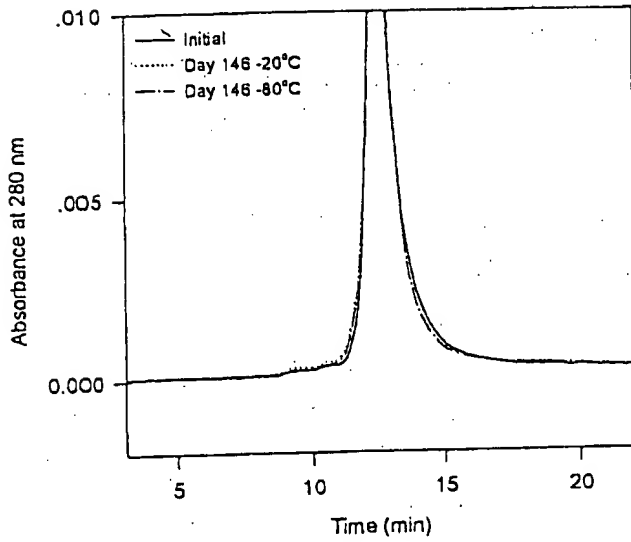
0.4 mg/ml AM424 in pH 4.5 Acetate Buffer



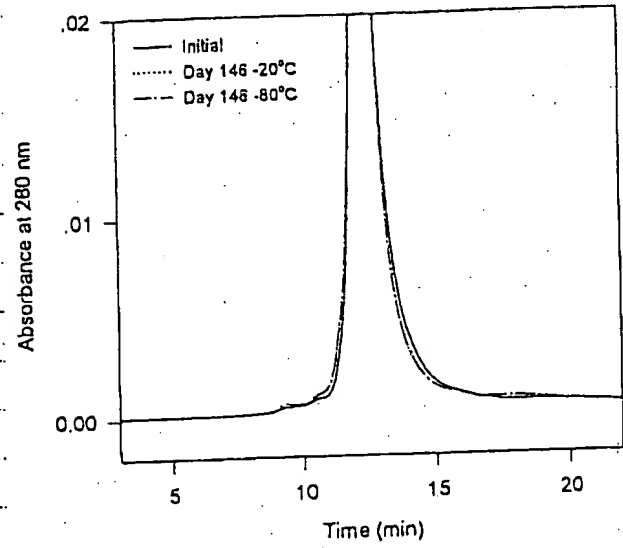
1.0 mg/ml AM424 in pH 4.5 Acetate Buffer



0.4 mg/ml AM424 in pH 5.0 Citrate Buffer



1.0 mg/ml AM424 in pH 5.0 Citrate Buffer



## LIF STABILITY STUDY AT 8°C AND 25°C

To observe change in LIF over 55 days stored at 8° and 25° analysing every 6-7 days.

### Solutions

#### HPLC Buffers

RP, IEC and SEC buffers prepared the same as for (page 83)

#### Sample Buffers

11.22 mm Acetate buffer pH 4 and pH 4.5

11.22 mm Citrate buffer pH 5

13.75 mm Acetate buffer pH 4.5

13.75 mm Citrate buffer pH 5

(all buffers contain 5% sorbitol and 0.01% tween 80 final concentration after LIF added)

ie: 11.22 mm buffers - 5.61% sorbitol, 0.011% tween

13.75 mm buffers - 6.87% sorbitol, 0.014% tween

same as for (page 83)

### HPLC

methods for RP, IEC and SEC same as for (page 83)

### Sterile Procedures

Follow same sterile procedures as for (page 83)

Method:

## LIF STABILITY STUDY AT 8°C AND 25°C

1. Add 32.08 ml of buffer - 11.22 mM Acetate pH 4  
 11.22 mM Acetate pH 4.5  
 11.22 mM Citrate pH 5  
 (3 x 10ml + 2ml + 80 $\mu$ l)

Add 26.19 ml of buffer - 13.75 mM Acetate pH 4.5  
 13.75 mM Citrate pH 5  
 (2 x 10 ml + 6 ml + 190 $\mu$ l)

to 5 sterile 50 ml tubes

2. Thaw 2 x 60 mg + 1 x 5mg of stock LIF ~ 40ml
3. Add LIF to each tube - 3 x 3 ml with 10ml graduated pipette then 3 x 920 $\mu$ l with pipetman for 0.4 mg/ml  
 - 2 x 9 ml with 10ml graduated pipette then 2 x 810 $\mu$ l with pipetman for 1.0 mg/ml

	LIF	Buffer	Total
0.4 mg/ml	3.92 ml	32.08 ml	36 ml
1.0 mg/ml	9.81 ml	26.19 ml	36 ml
total	31.38 ml		

4. Measure and record the pH of each formulation.

Formulation	Theoretical pH	Measured pH
0.4mg/ml LIF/Acetate	4.00	4.03
0.4mg/ml LIF/Acetate	4.50	4.52
0.4mg/ml LIF/Citrate	5.00	5.02
1.0mg/ml LIF/Acetate	4.50	4.54
1.0mg/ml LIF/Citrate	5.00	5.00

5. Sterile filter each formulation with a 50ml syringe into another sterile 50ml tube, discard the first 1.15 ml through the filter into a vial and cap and label it.
6. For each formulation draw up 12.5 ml in the multi-dispensing pipette and deliver 1.0 ml to 29 vials. Remove the 12.5 ml tip and put the 2.5 ml tip on and draw up 2.5 ml. Add 150µl to each of the 29 vials.
7. Cap, crimp and label all the vials. Put aside 2 vials from each formulation for Time 0 analysis.
8. Place racks containing remaining vials in plastic bags and put half the vials at 8°C and half at 25°C.
9. Analysis:
 

	Blank
	Standards 0.2, 0.4, 0.7, 1.0 mg/ml
0.4 mg/ml	Acetate pH 4 (1) & (2) 8°C
	Acetate pH 4 (1) & (2) 25°C
	Acetate pH 4.5 (1) & (2) 8°C
	Acetate pH 4.5 (1) & (2) 25°C
	Citrate pH 5 (1) & (2) 8°C
	Citrate pH 5 (1) & (2) 25°C
1.0 mg/ml	Acetate pH 4.5 (1) & (2) 8°C
	Acetate pH 4.5 (1) & (2) 25°C
	Citrate pH 5 (1) & (2) 8°C
	Citrate pH 5 (1) & (2) 25°C
	Blank
	Standards 0.2, 0.4, 0.7, 1.0 mg/ml

for each time point

\* For time 0, only 2 vials from each formulation analysed.

### Standards

For each time point thaw 1 nuc tube of stock LIF (330µl) for standards

0.2mg/ml - 450µl = 24.5µl LIF + 425.5µl buffer  
 0.4 mg/ml - 450µl = 49µl LIF + 401µl buffer  
 0.7 mg/ml - 450µl = 35.8µl LIF + 364.2µl buffer  
 1.0 mg/ml - 450µl = 122.6µl LIF + 327.4µl buffer

RESULTS:

pH								
SAMPLE	Nº	DAY 0	DAY 13	DAY 19	DAY 27	DAY 41	DAY 5	
AC4 0.4 8°	1	4.03	4.05	4.11	4.06	4.07	4.16	
	2		4.08	4.10	4.09	4.06	4.16	
AC4 0.4 25°	1		4.07	4.06	4.05	4.09	4.15	
	2		4.06	4.07	4.09	4.09	4.12	
AC4.5 0.4 8°	1	4.52	4.53	4.56	4.56	4.51	4.58	
	2		4.53	4.56	4.56	4.49	4.5	
AC4.5 0.4 25°	1		4.52	4.51	4.51	4.53	4.51	
	2		4.53	4.51	4.53	4.53	4.5	
CIT5 0.4 8°	1	5.02	5.03	5.08	5.06	5.03	5.0	
	2		5.04	5.08	5.06	5.04	5.0	
CIT5 0.4 25°	1		5.05	5.04	5.05	5.06	5.0	
	2		5.05	5.04	5.05	5.06	5.0	
AC4.5 1.0 8°	1	4.54	4.56	4.59	4.55	4.55	4.61	
	2		4.58	4.60	4.57	4.54	4.61	
AC4.5 1.0 25°	1		4.57	4.57	4.57	4.57	4.5	
	2		4.58	4.58	4.58	4.59	4.57	
CIT5 1.0 8°	1	5.00	5.05	5.08	5.02	5.04	5.0	
	2		5.06	5.07	5.03	5.04	5.0	
CIT5 1.0 25°	1		5.06	5.05	5.05	5.06	5.0	
	2		5.07	5.05	5.05	5.06	5.0	

Particle Size

Particle size was measured for each buffer (11.22mM acetate pH4, 4.5 citrate pH5 and 13.75mM acetate pH4.5 citrate pH5) and for each LIF sample at 8° and 25° on day 41 on a Malvern Instruments Zetasizer 3000 at 25°C. Each buffer and all the samples contained 0 particles.

pH	AC4 0.4		AC4.5 0.4		CIT5 0.4		AC4.5 1.0		CIT5 1.0	
	8°	25°	8°	25°	8°	25°	8°	25°	8°	25°
D120					5.10	5.10			5.08	5.1

# Results

LIF 0.4mg/ml/Acetate Buffer pH4.0

Table 1. Summary of 0.4 mg/ml LIF Stability at pH 4.0 at 8°C and 25°C

pH	buffer	LIF Conc. (mg/ml)	Storage Temp. (C)	Storage Time (days)	RP - Measured Conc. (mg/ml)	RP - Main Peak (area %)	IEC - Measured Conc. (mg/ml)	IEC - Main Peak (area %)	SEC - Measured Conc. (mg/ml)	SEC - Main Peak (area %)
4.0	acetate	0.4	8	0	0.40, 0.39	100, 100	0.37, 0.37*	98.9, 99.0	0.39, 0.40	98.8, 98.7
				7	0.40, 0.40*	100, 100	0.35, 0.34*	91.7, 87.2	0.39, 0.40*	97.6, 97.5
				13	0.39, 0.39	100, 100	0.33, 0.37*	90.8, 92.8	0.40, 0.40	98.9, 98.9
				19	0.40, 0.40*	100, 100	0.34, 0.33	89.7, 86.8	0.38, 0.38	98.8, 99.0
				27	0.40, 0.40*	100, 100	0.33, 0.33	84.6, 83.7	0.40, 0.40	98.9, 98.9
				41	0.40, 0.40	100, 100	0.34, 0.35	86.9, 88.2	0.40, 0.41	98.9, 98.9
				55	0.40, 0.41	100, 100	0.34, 0.33	89.2, 83.0	0.40, 0.40	99.0, 99.0
4.0	acetate	0.4	25	0	0.40, 0.39	100, 100	0.37, 0.37*	98.9, 99.0	0.39, 0.40	98.8, 98.7
				7	0.39, 0.39	100, 100	0.33, 0.36*	85.1, 91.5	0.39, 0.40*	97.3, 97.4
				13	0.40, 0.39	100, 100	0.28, 0.30*	74.7, 80.7	0.39, 0.41	99.2, 99.1
				19	0.40, 0.39	100, 100	0.31, 0.32	78.3, 80.3	0.38, 0.38	99.0, 99.2
				27	0.40, 0.40*	100, 100	0.29, 0.30	73.3, 74.5	0.40, 0.40	99.4, 99.2
				41	0.40, 0.40	100, 100	0.31, 0.31	76.1, 77.8	0.41, 0.41	99.2, 99.2
				55	0.41, 0.40	100, 100	0.25, 0.24	62.6, 59.8	0.40, 0.40	99.3, 99.7

## 0.4 mg/ml LIF IN ACETATE BUFFER pH 4.0

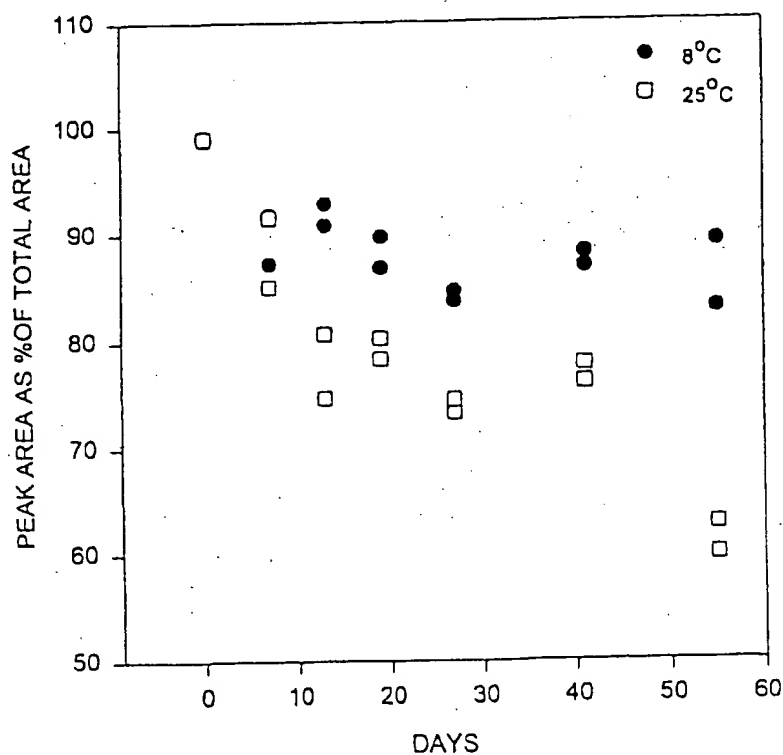




Table 3. Summary of 0.4 mg/ml LIF Stability at pH 4.5 at 8°C and 25°C

pH	buffer	LIF Conc. (mg/ml)	Storage Temp. (C)	Storage Time (days)	RP - Measured Conc. (mg/ml)	RP - Main Peak (area %)	IEC - Measured Conc. (mg/ml)	IEC - Main Peak (area %)	SEC - Measured Conc. (mg/ml)	SEC - Main Peak (area %)
4.5	acetate	0.4	8	0	0.39, 0.39	100, 100	0.36, 0.36*	99.0, 98.9	0.39, 0.38	98.8, 98.8
				7	0.38, 0.38	100, 100	0.37, 0.36*	95.4, 95.6	0.38, 0.39*	97.7, 97.8
				13	0.38, 0.38	100, 100	0.38, 0.38	98.3, 95.6	0.39, 0.38	99.0, 99.0
				19	0.38, 0.38	100, 100	0.37, 0.35	97.8, 93.3	0.38, 0.38	98.2, 98.2
				27	0.38, 0.38*	100, 100	0.35, 0.38	90.8, 94.1	0.39, 0.39	98.9, 98.8
				41	0.39, 0.39	100, 100	0.37, 0.38	95.3, 94.2	0.39, 0.39	98.9, 98.8
4.5	acetate	0.4	25	55	0.40, -	100, -	0.35, 0.33	89.6, 85.9	0.39, 0.39	99.0, 98.9
				0	0.39, 0.39	100, 100	0.36, 0.36*	99.0, 98.9	0.39, 0.39	98.8, 98.8
				7	0.38, 0.38	100, 100	0.36, 0.34*	94.7, 88.8	0.39, 0.39*	98.1, 98.2
				13	0.39, 0.38	100, 100	0.33, 0.35	86.8, 91.0	0.39, 0.38	99.0, 99.0
				19	0.38, 0.38	100, 100	0.31, 0.30	82.0, 80.0	0.38, 0.38	99.1, 99.0
				27	0.38, 0.38*	100, 100	0.30, 0.29	75.8, 73.5	0.39, 0.39	99.1, 99.2
				41	0.40, 0.40	100, 100	0.28, 0.28	71.2, 71.1	0.39, 0.39	99.2, 99.3
				55	0.39, 0.40	100, 100	0.22, 0.24	53.4, 59.1	0.39, 0.39	99.3, 99.4

## LIF IN ACETATE BUFFER pH 4.5

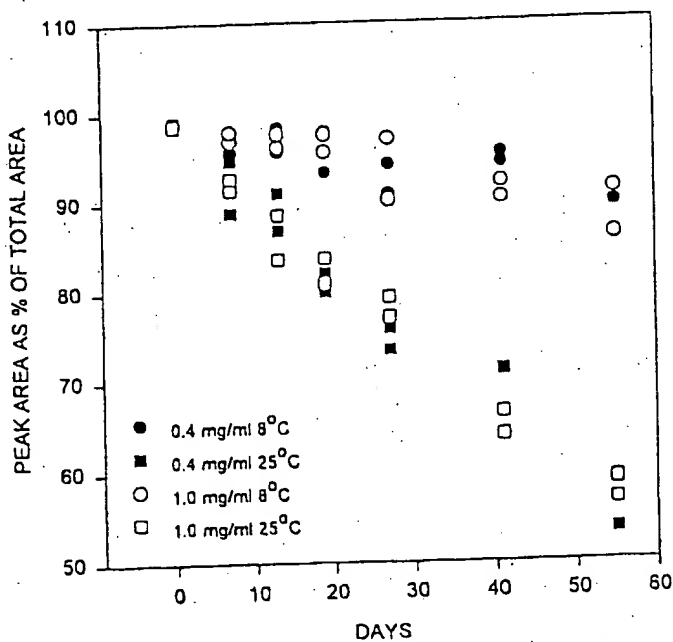


Table 5. Summary of 1.0 mg/ml LIF Stability at pH 4.5 at 8°C and 25°C

pH	buffer	LIF Conc. (mg/ml)	Storage Temp. (C)	Storage Time (days)	RP - Measured Conc. (mg/ml)	RP - Main Peak (area %)	IEC - Measured Conc. (mg/ml)	IEC - Main Peak (area %)	SEC - Measured Conc. (mg/ml)	SEC - Main Peak (area %)
4.5	acetate	1.0	8	0	0.99, 0.99	100, 100	0.98, 0.98*	98.5, 98.8	0.99, 0.99	98.6, 98.2
				7	0.98, 0.98*	100, 100	0.98, 0.99*	96.9, 97.9	0.99, 0.99*	98.8, 98.6
				13	0.98, 0.99	100, 100	0.94, 0.95*	96.2, 97.8	0.98, 0.98	98.8, 98.7
				19	0.98, 1.0	100, 100	0.98, 0.94	97.5, 95.6	1.0, 0.99	98.8, 98.8
				27	0.99, 1.0	100, 100	0.94, 0.88*	97.0, 90.1	1.0, 1.0	98.6, 98.8
				41	0.98, 0.99	100, 100	0.88, 0.90	90.3, 92.1	0.98, 0.98	98.9, 98.9
4.5	acetate	1.0	25	55	0.99, 1.0	100, 100	0.90, 0.85	91.2, 86.1	0.99, 0.99	98.9, 98.9
				0	0.99, 0.99	100, 100	0.96, 0.96*	98.5, 98.8	0.99, 0.99	98.6, 98.2
				7	0.99, 0.99*	100, 100	0.92, 0.94*	91.4, 92.7	0.99, 0.99*	98.9, 98.9
				13	1.0, 0.99	100, 100	0.82, 0.86*	83.8, 88.6	0.98, 0.98	99.0, 99.0
				19	1.0, 1.0	100, 100	0.84, 0.81	83.7, 80.9	1.0, 1.0	98.9, 98.9
				27	1.0, 1.0	100, 100	0.78, 0.81	77.1, 79.3	1.0, 1.0	99.0, 99.0
				41	0.99, 0.99	100, 100	0.68, 0.65	66.4, 63.9	0.98, 0.98	98.9, 99.1
				55	1.0, 0.99	100, 100	0.59, 0.60	55.7, 58.9	0.99, 0.99	99.1, 99.1

LIF 0.4mg/ml and 1.0mg/ml / Citrate Buffer pH 5.0

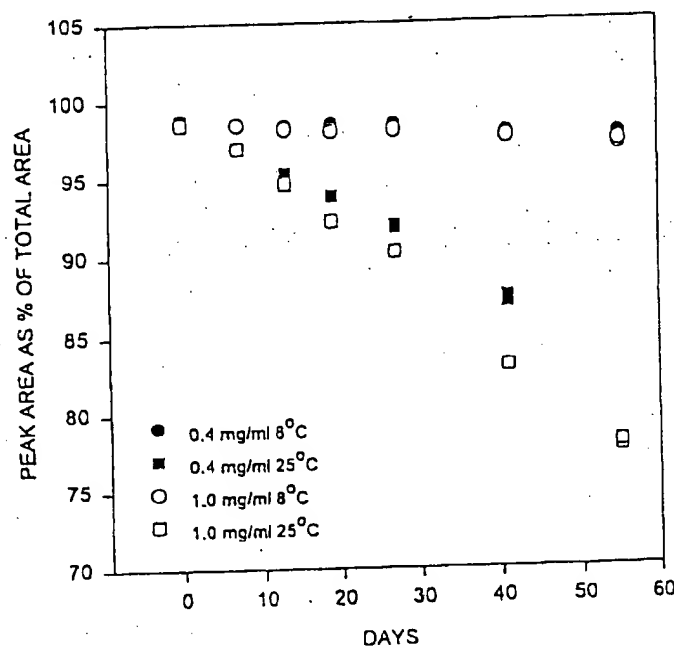
REDACTED

20

Table 7. Summary of 0.4 mg/ml LIF Stability at pH 5.0 at 8°C and 25°C

pH	buffer	LIF Conc. (mg/ml)	Storage Temp. (C)	Storage Time (days)	RP - Measured Conc. (mg/ml)	RP - Main Peak (area %)	IEC - Measured Conc. (mg/ml)	IEC - Main Peak (area %)	SEC - Measured Conc. (mg/ml)	SEC - Main Peak (area %)
5.0	citrate	0.4	8	0	0.38, 0.37	100, 100	0.36, 0.36*	98.8, 98.7	0.38, 0.38	98.8, 98.4
				7	0.37, 0.37*	100, 100	0.38, 0.38	98.4, 98.4	0.37, 0.37*	98.1, 98.1
				13	0.37, 0.37	100, 100	0.38, 0.38	98.4, 98.4	0.38, 0.38	98.8, 98.7
				19	0.37, 0.37	100, 100	0.37, 0.37	98.4, 98.5	0.37, 0.37*	98.3, 98.0
				27	0.38, 0.38*	100, 100	0.38, 0.37*	98.4, 98.5	0.38, 0.38	98.8, 98.6
				41	0.39, 0.39	100, 100	0.38, 0.37	97.8, 97.9	0.38, 0.38	98.7, 98.7
				55	0.39, 0.39	100, 100	0.37, 0.37	97.7, 97.5	0.39, 0.38	98.8, 98.7
5.0	citrate	0.4	25	0	0.38, 0.37	100, 100	0.36, 0.36*	98.8, 98.7	0.38, 0.38	98.6, 98.4
				7	0.37, 0.37	100, 100	0.37, 0.36*	97.0, 97.0	0.38, 0.38*	98.7, 98.5
				13	0.38, 0.37	100, 100	0.38, 0.37	95.4, 95.1	0.37, 0.37*	98.7, 98.7
				19	0.37, 0.37	100, 100	0.35, 0.35	93.8, 93.9	0.38, 0.38	98.8, 98.8
				27	0.38, 0.38*	100, 100	0.34, 0.34*	92.0, 91.8	0.39, 0.39	99.3, 99.0
				41	0.39, 0.39	100, 100	0.33, 0.34	87.0, 87.4	0.38, 0.38	99.1, 99.1
				55	0.39, 0.39	100, 100	0.30, 0.30	77.8, 77.8	0.39, 0.39	99.0, 98.9

LIF IN CITRATE pH 5.0



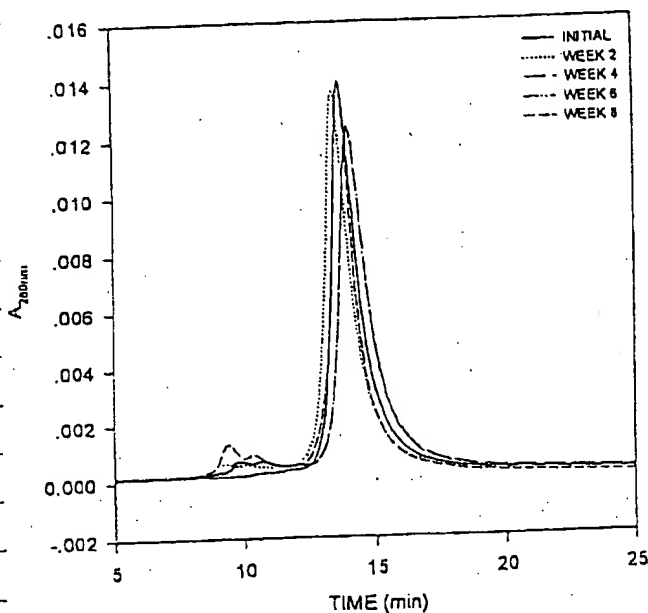
IEC

Table 9. Summary of 1.0 mg/ml LIF Stability at pH 5.0 at 8°C and 25°C

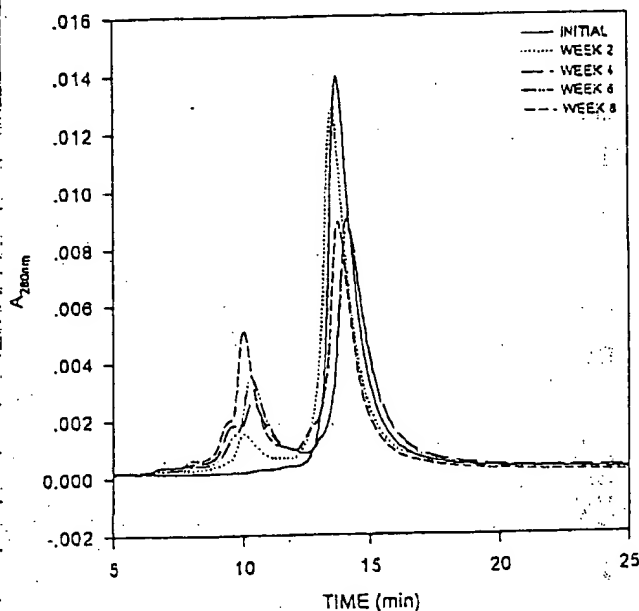
pH	buffer	LIF Conc. (mg/ml)	Storage Temp. (C)	Storage Time (days)	RP - Measured Conc. (mg/ml)	RP - Main Peak (area %)	IEC - Measured Conc. (mg/ml)	IEC - Main Peak (area %)	SEC - Measured Conc. (mg/ml)	SEC - Main Peak (area %)
5.0	citrate	1.0	8	0	0.98, 0.98	100, 100	0.95, 0.95*	98.5, 98.5	0.97, 0.97	98.2, 98.1
				7	0.98, 0.98*	100, 100	0.99, 0.99*	98.5, 98.5	0.97, 0.98*	98.5, 98.5
				13	0.97, 0.97	100, 100	0.94, 0.94*	98.1, 98.2	0.98, 0.98*	98.2, 98.0
				19	0.99, 0.99	100, 100	0.95, 0.95	98.1, 98.0	0.98, 0.98	98.5, 98.6
				27	0.98, 0.99	100, 100	0.99, 0.98	98.0, 98.1	0.98, 0.98	98.6, 98.6
				41	0.98, 0.98	100, 100	0.94, 0.94	97.5, 97.6	0.95, 0.96	98.7, 98.6
				55	0.98, 0.98	100, 100	0.94, 0.94	97.0, 97.2	0.97, 0.98	98.6, 98.8
5.0	citrate	1.0	25	0	0.98, 0.98	100, 100	0.95, 0.95	98.5, 98.5	0.97, 0.97	98.2, 98.1
				7	0.97, 0.97*	100, 100	0.97, 0.97*	97.0, 97.0	0.98, 0.98*	98.8, 98.6
				13	0.98, 0.97	100, 100	0.92, 0.91	94.8, 94.7	0.97, 0.97*	98.8, 98.8
				19	0.99, 1.0	100, 100	0.90, 0.89	92.2, 92.3	0.98, 0.98	98.8, 98.6
				27	0.99, 0.99	100, 100	0.91, 0.91*	90.3, 90.3	0.99, 0.98	98.8, 98.8

Chromatograms from Ion Exchange analysis  
for LIF 0.4mg/ml and 1.0mg/ml in Acetate Buffer pH 4  
(2 week intervals)

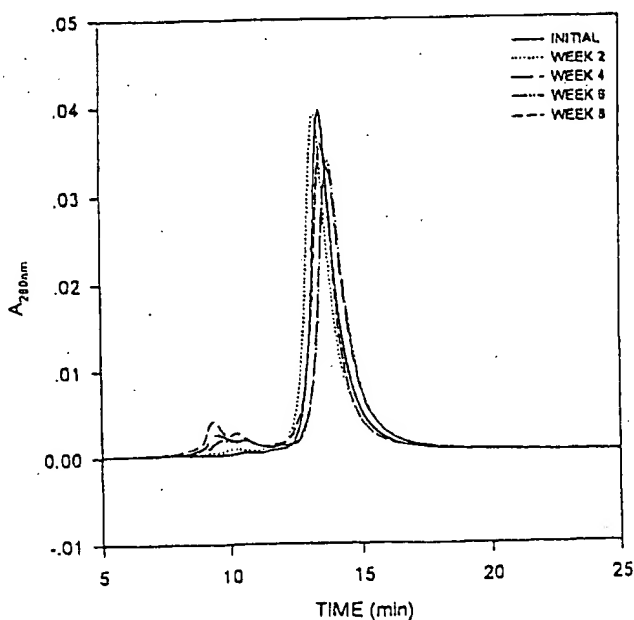
0.4 mg/ml LIF IN ACETATE BUFFER pH 4.5 AT 8°C



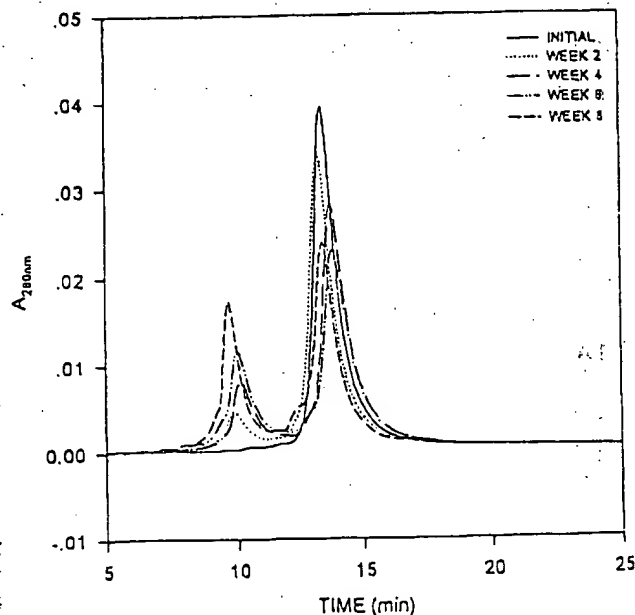
0.4 mg/ml LIF IN ACETATE BUFFER pH 4.5 AT 25°C



1.0 mg/ml LIF IN ACETATE BUFFER pH 4.5 AT 8°C

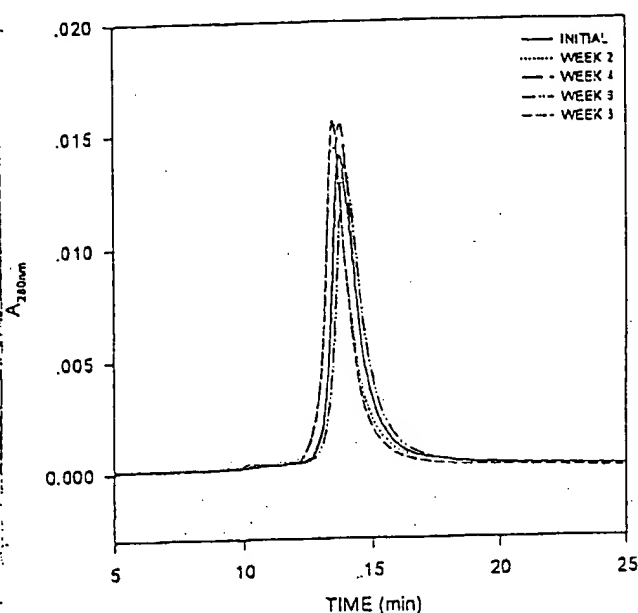


1.0 mg/ml LIF IN ACETATE BUFFER pH 4.5 AT 25°C

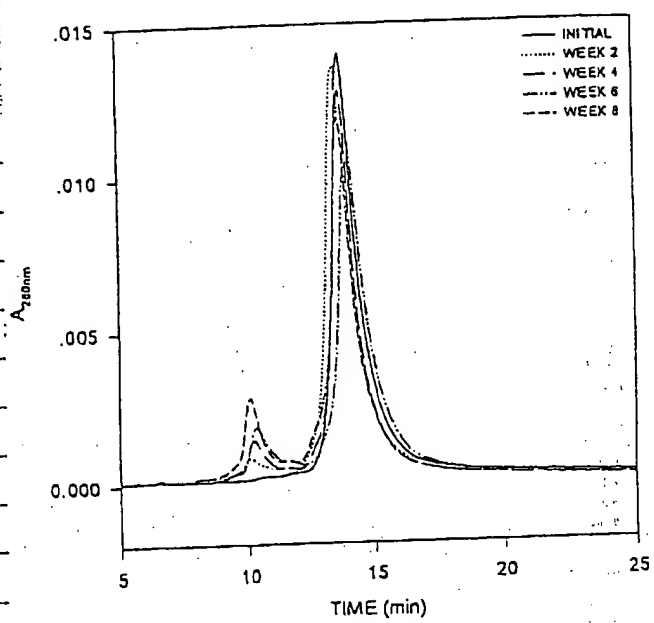


Chromatograms from Ion Exchange analysis  
of LIF 0.4mg/ml and 1.0mg/ml in citrate Buffer pH 5.0  
(2 week intervals)

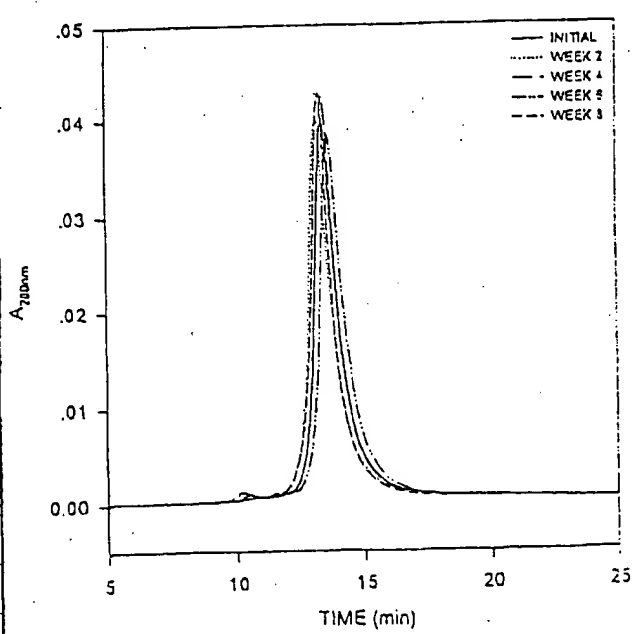
0.4 mg/ml LIF IN CITRATE BUFFER pH 5.0 AT 8°C



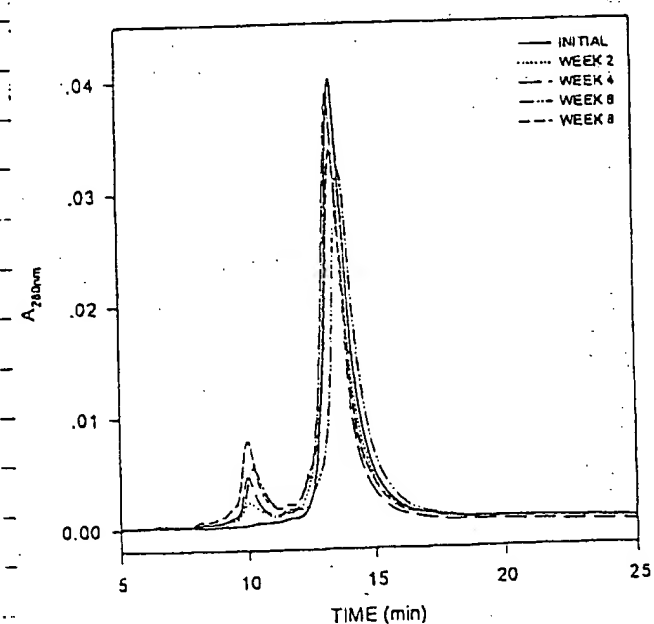
0.4 mg/ml LIF IN CITRATE BUFFER pH 5.0 AT 25°C



1.0 mg/ml LIF IN CITRATE BUFFER pH 5.0 AT 8°C



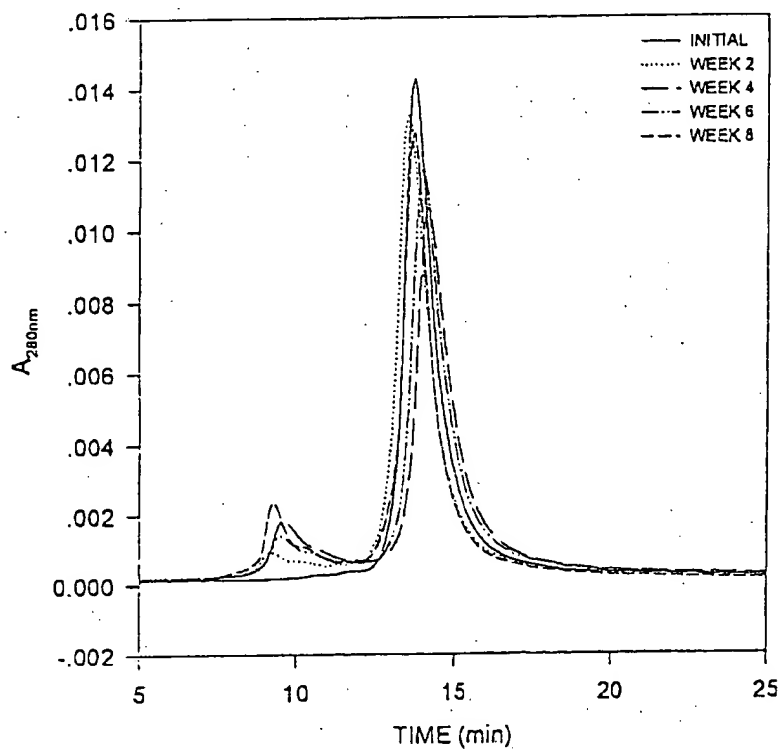
1.0 mg/ml LIF IN CITRATE BUFFER pH 5.0 AT 25°C



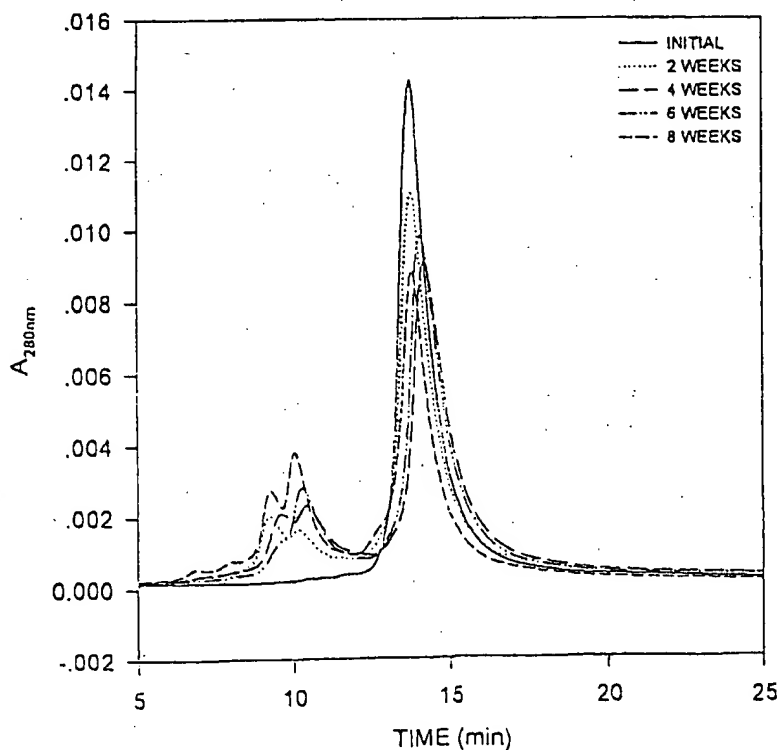
Chromatograms from Ion Exchange analysis of LIFO (1mg/ml) in Acetate  
buffer pH 4.0

0.4 mg/ml LIF IN ACETATE BUFFER pH 4.0 AT 8°C

(2 week  
intervals)



0.4 mg/ml LIF IN ACETATE BUFFER pH 4.0 AT 25°C



LIF in citrate buffer pH 5.0 showed much less degradation at 8° and 25° than the LIF in acetate buffer pH 4.0 and pH 4.5.

Over 55 days at 8° LIF in acetate showed a 13% (pH 4) + 12% (pH 4.5) loss of LIF

compared to 1% loss of LIF in citrate (pH 5)

At 25° LIF in acetate showed a 39% (pH 4)

and 43% (pH 4.5) loss of LIF compared to 20%

loss of LIF in citrate (pH 5).

There was little difference in the % loss of

LIF between 0.4mg/ml and 1.0mg/ml formulations

Acetate pH 4.5 - 8° = 12% (0.4mg/ml) 10% (1.0mg/ml)

25° = 43% (0.4mg/ml) 39% (1.0mg/ml)

Citrate pH 5 - 8° = 1% (both 0.4mg/ml and 1.0mg/ml)

25° = 21% (0.4mg/ml) and 20% (1.0mg/ml)

\* see final report 'Preliminary Stability Assessment of Prototype Solution Formulations for AM424'